Carbon Dioxide Rises Beyond Acceptable Safety Levels in Children Under Nose and Mouth Covering: Results of an Experimental Measurement Study in Healthy Children

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This is the long version of a study published previously as a short research letter by JAMA Pediatrics, but retracted (Walach, H., Weikl, R., Prentice, J., Diemer, A., Traindl, H., Kappes, A., & Hockertz, S. (2021). Retracted: Experimental assessment of carbon dioxide content in inhaled air with or without face masks in healthy children: A randomized clinical trial. *JAMA Pediatrics*. doi:10.1001/jamapediatrics.2021.2659). In our view, the retraction was not warranted and violated the ethical code of publishing, which knows three reasons for retractions, none of which was present: fabricated or wrong data, wrong analysis, plagiarism. The texts of our comments and rebuttals were not published by the journal, but can be found on Retraction Watch (https://retractionwatch.com/2021/07/16/jama-journal-retracts-paper-on-masks-for-children/). The “additional scientific review” JAMA Pediatrics mentions in the retraction was not sent to us despite our asking for it twice.

We therefore publish here the full text, as it was submitted to another peer-reviewed journal, where it is currently under review. The text might change as a consequence of peer-review.

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Key Points

Question: We wanted to know whether carbon dioxide levels in inhaled air rises beyond acceptable levels under mouth and nose covering (MNC)/face masks in children.

Findings: In an experimental, intra-individually controlled measurement study in 45 healthy children we found a highly significant rise from 2.700 parts per million (ppm) CO_2 at baseline to more than 13.000 ppm under surgical masks after only 1 minute, and to nearly 14.000 ppm under FFP2-masks.

Meaning: Wearing mouth and nose covering induces a rise in carbon dioxide levels in inhaled air in children that is beyond any accepted safety levels.

Abstract

Background: During the Covid-19 crisis many governments have made wearing mouth and nose covering (MNC) for children compulsory in schools and public transport. We do not know whether the carbon dioxide (CO_2) levels of inhaled air under MNC rises to levels that might be a health hazard for children.

Objective: To determine whether CO_2 levels in inhaled air rise with MNC and to what levels in short term measurements under two commonly used MNC types, surgical masks and FFP2 (N95) masks, compared to baseline.

Design: Experimental, intra-individually controlled study with a pre- and post-measurement baseline and randomized, counter-balanced application of two different MNC types, surgical and FFP2 (N95) masks. CO_2 content was measured every 15 seconds using an automated dual-wavelength infrared CO_2 measurement device (G100, Geotech, Leamington Spa, UK) over 25 minutes in a short-term experimental setting.

Setting: Private practice with stationary measurement set-up. Measurements were conducted in seated resting children.

Participants: 45 healthy children of school-going age between 6 and 17 years who volunteered and gave written informed consent.

Interventions: Application of two types of commonly worn MNC: surgical masks and FFP2 (N95) masks in randomized order for three minutes each.

Main Outcome Measure: Carbon dioxide (CO_2) content of inhaled air under baseline conditions and two types of MNC; CO_2 was also measured in exhaled and in the mixture of inhaled and exhaled air. As control variables we measured ambient CO_2 several times during a measurement and kept ambient CO_2 well below 1.000 parts per million (ppm) through frequent ventilation. We measured breathing frequency and pulse as potential moderator variables.

Results: Forty five children, 25 boys, 20 girls, between age 6 and 17, with a mean age of 10.7 years (standard deviation 2.6) were measured. We measured 2'678 ppm (SD 1'080) CO_2 at pre-baseline and 2'814 (SD 1'055) at post-baseline, a non-significant small difference. We measured 13'125 ppm (SD 3'836) under surgical mask and 13'913 ppm (SD 3'738) under FFP2 mask. A linear model with age as a covariate showed a highly significant change over time ($p < 1*10^{-9}$). Appropriate contrasts revealed that the change was due to the masks only and the difference between the two types of masks was small and not significant.

Conclusion and Relevance: Wearing of MNC (surgical masks or FFP2/N95 masks) raises CO_2 content in inhaled air quickly to a very high level in healthy children in a seated resting position. These results should prompt decision makers in schools and governments to reconsider orders for compulsory MNC, as CO_2 rises of such a magnitude might be hazardous to children's health and explain some of the frequently documented effects such as fatigue, headache, dizziness and irritation.

Key Words: Covid-19, face masks, children, carbon dioxide, breathing, CO_2, randomized study

Background

Since the WHO has alarmed the world to the SARS-CoV2 pandemic in March 2020 most governments try to stop the spread of the novel corona virus. The governments of Germany and Austria, and possibly in other countries as well, have begun to make the wearing of nose and mouth covering (NMC), or face masks, compulsory for children going to school. The evidence-base for such a procedure to prevent infection is mixed at best. Two recent systematic reviews reach the conclusion that wearing face masks does not prevent infections by influenza virus, which is very similar to SARS-CoV2 [1,2]. Only very few data support the wearing of NMC in general contexts, and practically none for children [3]. Perhaps wearing NMC is popular, because in Hong Kong and Taiwan it was possible to stem the infection rapidly and here 98% of the population was wearing NMCs in public [4]. A review of non-randomized studies concludes that a small benefit cannot be excluded [5]. However, the first pragmatic randomized study comparing the suggestion to wear NMC in public with no recommendation found that the effect is small and not significant[6]: of 6.000 participants 42 or 1.8% were infected in the experimental group, and 53 or 2.1% in the control group. When comparing those that actually did wear the masks the effect was even smaller. Positive effects of MNC for preventing infections in community settings are likely small and probably only useful in high incidence environments [7,8].

Against this background of a potential but unknown small protective benefit the question whether NMC increases carbon dioxide in breathed air is getting more important. The first large scale German survey in parents and children, the Co-Ki-study of the University Witten/Herdecke using data of 25.930 children has shown that children report side effects to a high percentage [9]: 68% of parents report that their children have problems. Most frequently they report irritation, tension and stress (60% of parents report this), headaches (53%), difficulties concentrating (50%), fatigue and sleepiness (30%). It is possible that a high content of carbon dioxide in breathed air might be causal for those symptoms and complaints.

The normal content of carbon dioxide in breathed air in the open is about 0.04 volume % (i.e. 400 parts per million/ppm). 0.2 vol% or 2.000 ppm are acceptable for closed rooms according to the German federal environmental office[10]. This is at the same time the cut off for children and pregnant women, which is considered safe. Maximum concentration at the working place for healthy adults during 8 hours of work and 40 hours per week is considered 0.5 vol% or 5.000 ppm.

To the best of our knowledge there are no solid peer-reviewed data on carbon dioxide concentration in breathed air under NMC, especially for children. There are two studies that measured end tidal CO_2 pressure ($PetCO_2$) in children wearing face masks using capnographs. One study measured 47 healthy children for 60 minutes, with and without exertion [11]. While there were no significant changes, $PetCO_2$ fell by 0,5 mm Hg in the younger children and by 1 mm Hg in the older children after mild exertion. The other study measured 106 children over a period of 45 minutes using two different masks and a scheme of mild exertion. They found a rise in $PetCO_2$ by 3.2 mm Hg, and a clinically relevant standardized mean difference (d) of 1 standard deviation in a resting condition and a rise by 3.8 mm Hg under slight exertion, equivalent to an effect size of d = 1.3. [12] While the outcome parameters were clinical safety limits which were not violated and distress signals which were not seen, this study shows that physiological parameters change. But none of the studies measured the actual carbon dioxide content in inhaled air under a face mask.

Ing. Dr. Traindl, coauthor of this study, has made some pilot measurements in 3 persons and found 3-5% CO_2 in breathed air under NMC (30.000 – 50.000 ppm). One of these volunteers was a child, and here CO_2-concentrations were steadily measured at 3,4-5,0 % (34.000-50.000 ppm) [13]. A team from South-Tyrol/Italy conducted measurements in November 2020 in 24 volunteers using different types of NMC and clarified discrepancies to a study that had been conducted by the official government of the autonomic region in Bolzano [14]. The results reported by Ing. Oberrauch are considerably higher than those reported by the government. This is obviously due to the fact, that the governmental working group of the region of Bolzano had subtracted the environmentally measured carbon dioxide values from measures, which led to an artificially lowered result. The data of the South Tyrolian study [14] regarding different types of NMC range from 3.143 ppm for baseline with no mask to 7.292 ppm for surgical masks and 15.000 ppm with FFP2. These were results with adults and a few children and adolescents, and to our knowledge no systematic data on CO_2 content in inhaled air under NMC exist for children.

This is the reason why we wanted to measure in a well-controlled, experimental study in volunteer children carbon dioxide content in inhaled air with and without different types of NMC to find out whether raised values are found under different conditions and how CO_2 content changes in inhaled air under NMC.

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Method

Participants

Participants were children at school age, whose parents have shown interest in the study and were willing to give consent for their children to participate. Children also gave their own consent. The children were healthy, free from infections or neurological diseases, had no psychological disorders that would produce problems during wearing a face mask and had no medically indicated exception from the compulsory NMC order for school children that was effective in Germany at the time of measurement.

Participation was strictly on a volunteer basis and no remuneration was given. An informed consent and information leaflet for children was presented and informed consent of children and their parents was sought. The study was approved by the Ethics Committee of the University Witten/Herdecke (Registration Number 22/2021).

Measurements and Design

We measured in a short-term experimental protocol how the CO_2-concentration in inhaled air in the facial area without NMC and under NMC developed. The main outcome was the carbon dioxide content of the inhaled air, both under normal conditions and under NMC condition. We also measured the CO_2-concentration in exhaled and mixed inhaled/exhaled air in NMC, and the CO_2-concentration in inhaled air without NMC (baseline). The design of the study was an intra-individually controlled experiment with a baseline measurement before and

after the experimental measures. Children wore a surgical mask and a FFP2 mask in randomized, balanced order. The measurements were taken by an automated device through a measurement tube fixed to the face of the child about 1,5 cm distant from the nostrils, between upper lip and nose, with a flexible band that was adapted to the head size of the child. The device (G100 CO_2 incubator analyzer, Geotech, Leamington Spa, UK) measures CO_2 content of the air via dual wavelength infrared measurement every second; measurement range (full scale): 0 - 20 vol.%; accuracy ± 1% of measurement range after calibration. The calibration by the manufacturer with standard gases states that in the measuring range of 0 - 5 vol % the device has a precision between 0.064 and 0.080 vol%, i.e. a precision of ± 0,1 vol% of the calibrated range. The response time of the CO_2 sensor is approximately 1 second. The response time of the whole system – sensor and measurement tube – is dependent on the length of the tube and was less than 20 seconds in our case.

The measurement tube remained in place throughout the measurements, which lasted approximately 25 minutes for each child. Apart from time for preparation, 3 minutes measurement were taken for baseline carbon dioxide in inhaled air without face mask. Nine minutes measurement for each type of mask were allowed, 3 minutes for measuring carbon dioxide content under the face mask in joint inhaled and exhaled air, 3 minutes for measuring carbon dioxide during inhalation and 3 minutes during exhalation. As carbon dioxide content rises through exhalation and its rapid diffusion is prevented by the fabric of the mask which accumulates the exhaled CO_2, it is important to know and to measure the amount of CO_2 in all types of air, especially in inhaled air.

During baseline, carbon dioxide during inhalation only was measured. During mask-wearing carbon dioxide joint mixed air was measured first. This is the air that is freshly inhaled and mixes with the exhaled air that accumulates in the dead space volume of the mask. Then carbon dioxide in inhaled air only was measured, and finally carbon dioxide in exhaled air only. The measurement of the respective breathing phases was initiated by a medical doctor (RW) who observed the breathing patterns of the child carefully, and triggered the aspiration mechanism, a pump that is integrated in the measurement device, once the target phase (inhalation, exhalation) began and ended the measurement, when the target phase was over. This assured that only the particular type o fair that was intended for measurement was collected in the measurement tube and was then forwarded to the measurement sensor.

As the measurement device aspires the air through a small tube to the analyzer, there was a delay of 15 seconds until the target sample reached the device. This time delay was taken into account, when defining different phases for the analysis of the measurements.

During the first 3 minutes under the face mask the mixture of inhaled and exhaled air that collects under the mask (called "joint air") was measured. Then, after a 30 second waiting period to allow for the adaptation of the system to the new measurement, CO_2 was measured exclusively during inhalation for another 3 minutes. And, after a second measurement break of 30 seconds, CO_2 was measured exclusively during exhalation. The face mask was changed, which took around another 30 seconds and the same sequence as before was carried out (see e-Figure 1 in the Supplement for a sample measurement protocol). Each child was provided with a fresh set of masks. Masks by different producers were used randomly to cover an adequate and practical range of masks used in the community and to avoid any potential producer bias (see e-Table 1 in the Supplement).

While the sequence of masks was counterbalanced and randomized, the sequence of the measurements for one condition was always CO_2 content in joint air first, then inhaled and

finally exhaled air. This sequence was kept in order to have a comparable time lag for each type of measurement per child.

The measurement protocol was published in advance and is available at the Open Science Foundation platform at https://osf.io/yh97a/?view_only=df003592db5c4bd1ab183dad8a71834f.

During each experiment the room was well ventilated several times and control measurements were taken of the CO_2 content of ambient air several times during each measurement with a second measurement device (PCE-CMM 10 by PCE; measurement range 0ppm – 5.000ppm [0-0,5vol%], resolution 1ppm). CO_2 content was always kept well under 1.000 ppm or 0,1 volume %.

Measurement Protocol

First, measurements of CO_2 content in inhaled ambient air without MNC were taken (3 minutes, baseline 1). This was followed by measurements of air under masks. The sequence of masks was randomized and counterbalanced. Measurement of air under one mask took approximately 9 minutes per mask, 3 minutes for each of the 3 different measurement types (joint, inhaled, exhaled air). Finally, a 3-minute post-baseline measurement without mask concluded the protocol. At the final minute of each MNC-measurement block pulse and breathing frequency were measured, as well as blood oxygenation. There were the following deviations from the original protocol which were due to simplifications and time restraints: The experimental measurements were approximately 18 minutes in length instead of 15. The blood oxygenation measurements were not carried on after the measurements of the first children had revealed that blood oxygenation never dropped below 98% and was nearly always at 99%, making this variable superfluous. We did not carry out measurements of temperatures and of the breathing volume, as we did not expect reliable results with a face mask. Also, anticipated measurements of the breathability of the material were not done, as these were intended for potential “community masks” which were not used.

Controls, Randomization and Quality Assurance

Blinding was considered unnecessary, as the measurements are objective. Measurements were conducted exclusively with calibrated and producer-certified apparatuses. The measuring engineer has ample experience in using the apparatuses and has conducted a pilot study. He is a court-certified, oathbound authorized expert for the measurement of the burden of indoor air with carbon dioxide and methane. Data were documented in real time by written documentation and data capture via the instruments used (data tracing, screen snap shots). Although the device took measures every second, we used only measurements every 15 seconds, because this assured that the whole period of one experiment of 25 minute duration could be documented on one screen (see e-Figure 1). The data of one sequence of 3 minutes measurement (i.e. joint air, inhaled air, exhaled air, 12 to 15 measurements per sequence) were then averaged for statistical analysis.

The sequence of masks was randomized and randomization was stratified by age of children (below and above age 10). Randomization was conducted using randomizer.org. Two sets of random numbers were prepared, for children up to 10 years of age and older. A coin toss decided whether even or odd numbers meant first surgical or first FFP2 masks. Accordingly, cards with the sequence written on it were put in sealed opaque envelopes with sequential numbering of the child and the age category written on it. Hygiene rules were followed according to regulations. Personnel was tested to be free of SARS-CoV2.

Statistics – Power Analysis

Power Analysis

There were no pilot data available, hence we based our analysis on existing data [14]. We assumed that we will measure 3.000 ppm (or 0,3 vol%) CO_2 at baseline, i.e. a value which is slightly above current accepted norms because 1.000 ppm was expected to be ambient air and a higher value was expected because exhaled CO_2 remains in the vicinity of the face for a while. Thus, this is a conservative estimate. We assumed further masks will produce values between 5.000 ppm and 12.000 ppm CO_2 in inhaled air. The table of raw-data from [14] allowed us to calculate the mean for CO_2-content of breathed air without masks as 3.143 ppm, with surgical mask of 7.292 ppm, as well as a standard deviation of 2.500 ppm for surgical masks, and 1.000 ppm for no masks. This results in standardized mean differences (calculated with the larger SD for a conservative estimate) of d = 1.6. In order to secure such a strong effect with 90% power 7-9 children would have been sufficient per comparison, i.e. 18 children altogether. We used a safety factor of 2 and aimed at 40 to 50 children to be included.

Handling of Missing Data and Data Treatment

There were few missing data. In some cases, children stopped their experiment early and were not willing to allow for a post-measurement baseline. Such missing values were not interpolated. Sometimes a phase of measurement, for instance inhalation under surgical mask, was comparatively shorter than other phases or those of other children. However, in each and every case, there were enough data to calculate a phase-specific average. Data were averaged between the phases (baseline pre, mask 1 joint mixed air, mask 1 inhalation, mask 1 exhalation, mask 2 joint mixed air, mask 2 inhalation, mask 2 exhalation, baseline post).

Statistical Analysis

The statistical analysis used a linear model with a time-factor. As the mask-type was counterbalanced, a check was run whether there was a sequential effect using a simple t-test and visual inspection. There were no differences between the sequences, and hence the sequence was not entered into the model as categorical predictor. Preconditions of linear modeling were checked and met. Since some of the children were not able or willing to stay until the post-baseline measurement, this was discarded from further analysis, because the missing data would

have reduced power. There was no numerical and statistical difference between the baseline and the post-baseline (see e-Figure 3 in the Supplement). Correlations of predictors such as age, breathing frequency, pulse frequency, ambient CO_2 levels were inspected via scatterplots. The only potential predictor was age which was negatively correlated with CO_2 content of inhaled air, i.e. the CO_2 content was larger for younger children, and this was used as a covariate in the linear model (See Figure 1). All analyses were calculated using Statistica Version 13.3.

Results

Forty-five children or their parents called in to participate in the study. Due to organizational restraints – the experiment was tightly timed – and because after three days 45 participants, the figure stipulated in the protocol, was reached we stopped recruitment. No child was excluded because of a medical condition or exclusion criteria. Children were included in sequential order as they called in for the study. The mean age was 10.73 years (standard deviation 2,63; range 6 – 17). Twenty children were girls, 25 were boys.

Results are presented in Table 1. Figure 1 presents the correlation scatterplot of age vs. carbon dioxide under FFP2 masks.

Table 1 – CO2 values (vol%) under different conditions: means, (standard deviation), [95% confidence intervals], median, minima and maxima, n); * - main outcome

	Mean (SD)	Median	Minimum	Maximum
Baseline Pre (n=45)	0,268 (0,108) [0,235; 0,300]	0,230	0,1	0,628
Baseline Post (n = 39)	0,281 (0,105) [0,247; 0,316]	0,260	0,1	0,525
*Inhaled Surgical Mask (n = 45)	1,312 (0,384) [1,197; 1,427)	1,300	0,577	2,554
*Inhaled FFP2 (n = 45)	1,391 (0,374) [1,279; 1,504]	1,375	0,6	2,475
Joint Exhaled and Inhaled Surgical Mask (n = 45)	2,650 (0,486) [2,504; 2,796]	2,745	1,33	3,41
Exhaled Surgical Mask (n = 44)	3,847 (0,678) [3,641; 4,053]	4,116	1,783	4,754
Joint Inhaled and Exhaled FFP2 mask (n = 45)	2,677 (0,386) [2,561; 2,793]	2,745	1,66	3,418
Exhaled FFP2 (n = 45)	3,846 (0,547) [3,682; 4,011]	3,975	2,592	5,24
Ambient Air CO_2 Content	0,074 (0,003) [0,073; 0,075]	0,074	0,067	0,083

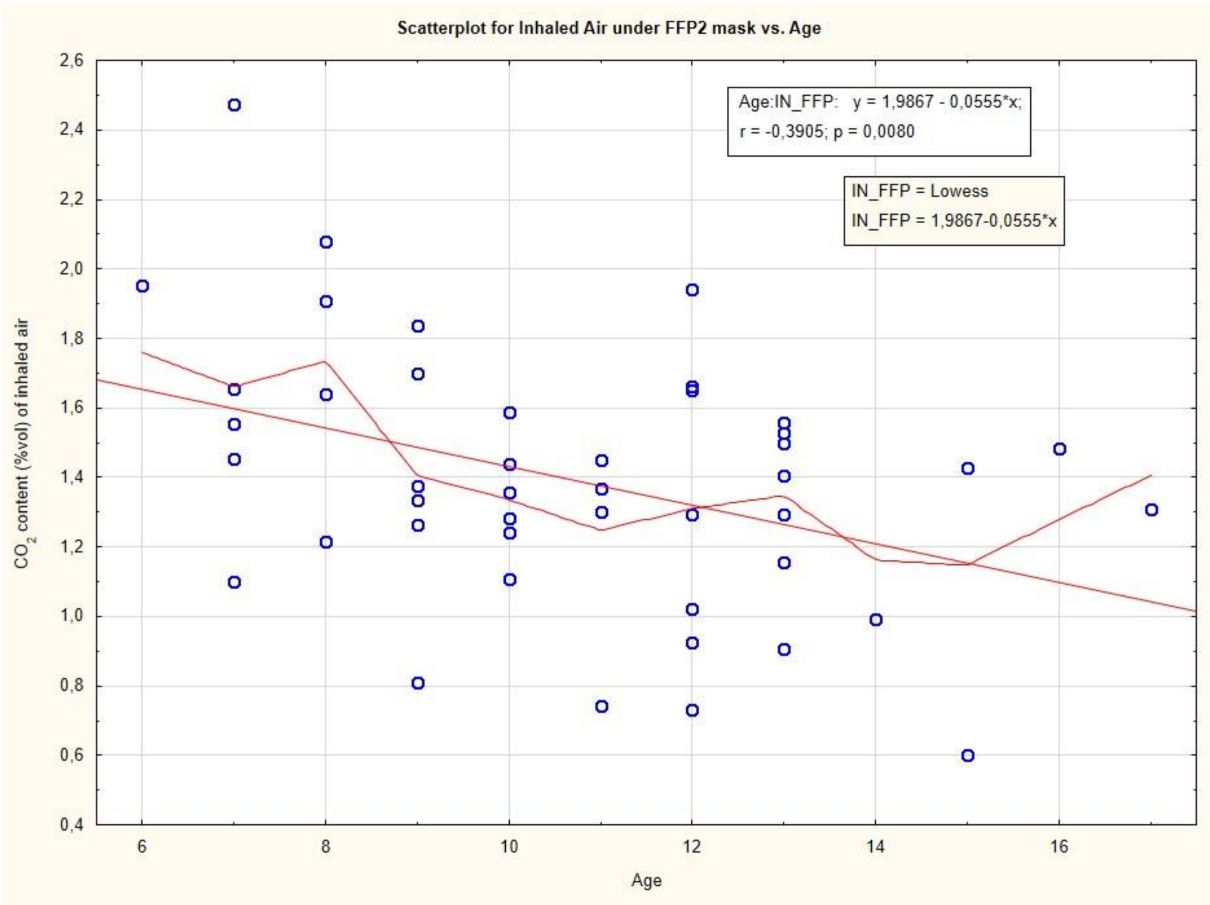

Figure 1 – Scatterplot of CO_2 content in inhaled air under FFP mask vs. Age with locally weighted scatterplot smoother to demonstrate approximate linearity

The linear model over time is presented in figure 2.

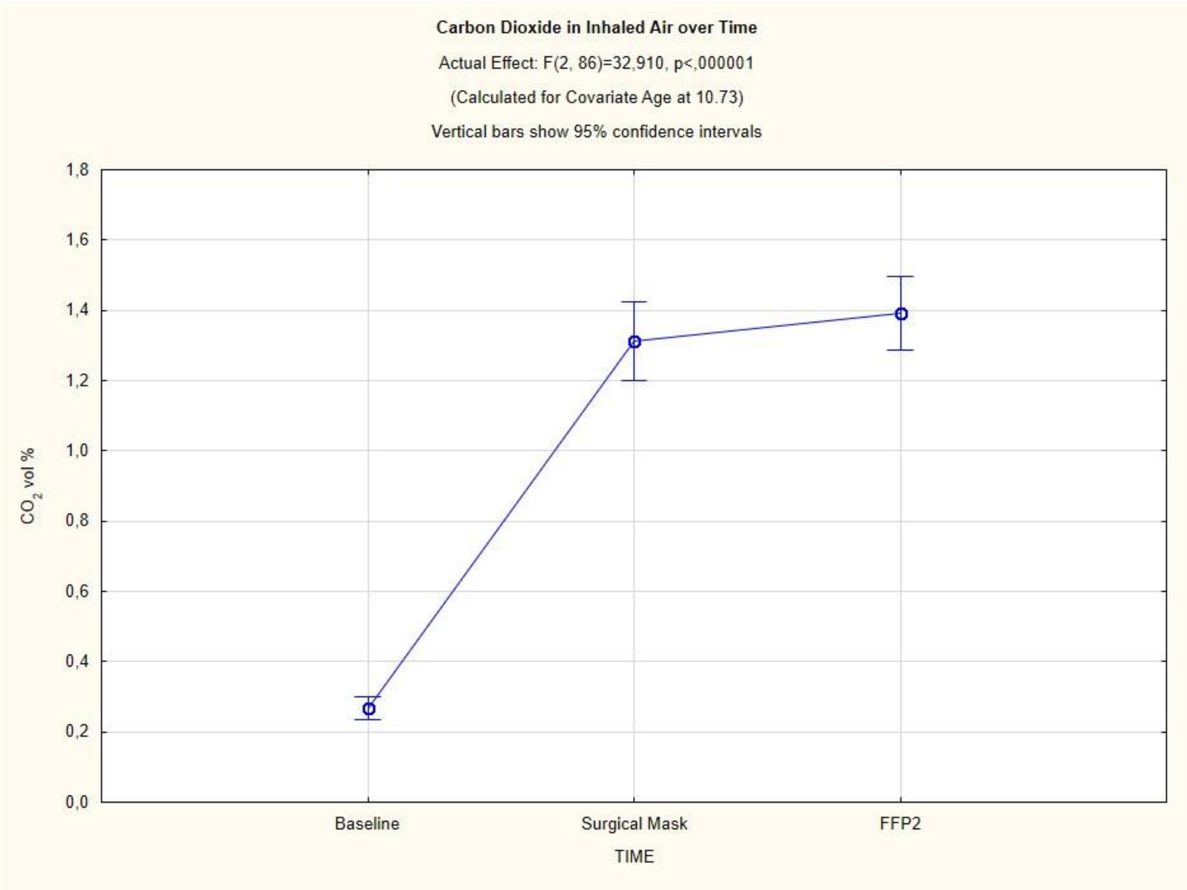

Figure 2 – Average carbon dioxide content (vol%) in inhaled air during baseline (no mask) and after 6 minutes breathing under a surgical and a FFP2 mask; results of a linear model with age as covariate

Linear modeling with age as a significant covariate (covariate age: $F = 5.6$; $p = .022$; partial $eta^2 = 0,11$; interaction age*time: $F = 4.09$; $p < .02$; partial $eta^2 = 0.08$) revealed a strong time effect ($F = 32.9$; $p < 1 * 10^{-9}$; partial $eta^2 = 0.43$). Contrasts showed that the effect is due to the difference between baseline and both masks jointly. Contrasts between the two types of masks were not significant ($F = 2,38$; $p = .13$). Residuals were normally distributed and the linearity assumption was met. Linear models of the other carbon dioxide measures - in exhaled air, in joint inhaled and exhaled air and an average of all three – reveal the same pattern of very steep rise from baseline and no difference between the two types of masks, with FFP2 masks showing slightly higher values and were all highly significant. E-Figure 4 represents this pattern from the data in Table 1 as a Box-and-Whiskers-Plot.

There were no significant effects in breathing frequency and in pulse, although a slight increase both in breathing frequency and pulse was visible (e-Table 2). Oxygen saturation of the blood remained always at 98-99%.

Discussion

The goal of this study was to find out, whether children breathing under a face mask – a surgical mask and an FFP2 mask – would be exposed to carbon dioxide levels in inhaled air beyond those assumed safe and not hazardous to health under current regulations in Germany. We deliberately used a still setting in which children were not exposed to any physical or mental workload that would increase their demand on oxygen supply. Even under conditions of

sitting still for approximately 9 minutes with each mask, we measured strong increases in the carbon dioxide of the inhaled air under the face mask. The increases were numerically large and statistically highly significant. The results were very robust.

A value of 5.000 parts per million (ppm) or 0,5% volume of CO_2 is considered the maximum exposure level of the German healthy at work regulations for workers during the day. An official statement of the German Federal Environmental office states that any value above 2.000 ppm (i.e. 0,2 vol%) in indoor air is “not acceptable”.[10]

We measured between 13.000 and 13.750 ppm of CO_2 in median in inhaled air under surgical and FFP2 masks, which is by a factor 6 higher than the 2.000 ppm that is already deemed “inacceptable” by the German Federal Environmental office. This limit of 2.000 ppm of CO_2 is by a factor 5 higher than the CO_2 content in normal air (400 ppm). What we measured is an average value of inhaled air during 3 minutes of measurement and after 6 minutes of wearing each mask. It is safe to assume that later measurements would have not produced lower values, although it would be interesting to learn what longer time monitoring would result in. Children under normal conditions in schools wear such masks for a much longer time span, often for hours. This high content of CO_2 in breathed air may explain why in a large German survey in more than 25.000 children 68% of the parents report impairments and problems [9]. Most of them can be understood as consequences of elevated CO_2 levels in air, which might lead to functional and physiological impairments.[15] A transcutaneous measurement study in medical personnel wearing surgical masks for 30 minutes confirmed that surgical masks lead to a re-inhalation of carbon dioxide and to an elevated partial pressure of CO_2 which is not compensated by altered breathing patterns.[16] Although we did see occasionally changed breathing, overall breathing frequency did not change during our experiment.

Why were our CO_2 measurements so high? We assume that the inhaled fresh air mixes with the exhaled air retained partially by the mask in its dead space volume and hence the CO_2 content of the inhaled air is successively increased until a comparatively high level is reached in a kind of novel steady state. This likely represents the exhaled CO_2 that is partially trapped in the dead space volume of the face mask and mixed with the fresh air coming in with each breath. This is supported by direct measurement study with similar results [16]

Two studies in children wearing NMC did not find clinically relevant altered end tidal CO_2 pressure[11,12]. One of them, however, found a relevant change of 3,2 mm Hg, or 3,8 mm Hg respectively, after 45 minutes wearing NMC. No study evaluated long term breathing physiology. We were not interested in capnography and physiological values, but in the actual CO_2 content of inhaled air. Relevant safety regulations use this parameter to determine safety levels, as ample evidence exists that exposure to CO_2 levels beyond 2’000 ppm poses a health risk[10]. The human organism seems to have enough buffering capacity for a while, until such elevated CO_2 levels translate into physiologically visible distress signals. We did not see changed breathing patterns or a decline in O_2-saturation short term. But the symptoms often reported by children [9] are consistent with subclinical symptoms of hypercapnia.[15] We note that even the child with the lowest CO_2 level in inhaled air (Figure 1) was by a factor 3 over the limit of 0,2 vol%, and the child with the highest CO_2 level in inhaled air was measured at 25.000 ppm, by a factor 12 higher than the limit.

It should also be noted that the goal of capnography and our measurements are completely different. While capnography is a safety monitoring tool to monitor breathing physiology under duress, such as anesthesia or in emergency, and can inform physicians about

critical situations, our goal was much more modest. We wanted to measure the CO_2 content in inhaled air under NMC. We leave it to further work to determine if and how such elevated CO_2 levels as we found is causally linked to symptoms of sub-clinical or clinical relevance.

A recent review summarizing 109 experimental studies, 44 of them quantitatively concluded that there was ample evidence for adverse effects of wearing face masks [8]. They are prone to induce what is now called mask induced exhaustion syndrome (MIES) with headache, fatigue, dizziness as its main symptoms, similar to what Schwarz and colleagues found [9]. Our findings are in support of this and can explain why: Even after a short period of time carbon dioxide rises under the mask to unacceptable levels. This is because the fabric prevents free exchange of air and because of the dead-space volume of the masks which collects the exhaled CO_2 and provides it for re-inhalation mixing it with fresh oxygen entering the mask through the fabric. The process is illustrated in e-Figure 4.

Our results are robust: We used average values of a series of measurements. We saw the same pattern in inhaled air, the important parameter, but also in exhaled and joint inhaled and exhaled air, measured by a calibrated and certified measurement apparatus. We counterbalanced masks, blinded randomization, stratified the randomization by age, and the analyses showed that there were no confounding effects of breathing frequency, mask sequence or other variables. We controlled the only potential covariate, age in our main model.

Limitations of our data can be considered the fact that we only measured sedentary children. Because of time restraints we could not conduct a more extended measurement with various conditions, such as physical exercise, or relaxed reading. Instead, all children were just measured seated. While some of them brought a book and read during the measurement, others simply observed the experiment. Further work might consider a more extended period of measurement time, real life monitoring or measurement after exertion. Also, long term measurements after prolonged mask-wearing after a full school day should be instantiated to see whether oxygen saturation of the blood is affected long term.

One might ask why our baseline measurements were comparatively high, although the ambient CO_2 content was kept well under 0,1 vol% and was on average 750 ppm. This is due to the fact that we measured in the vicinity of the face, and exhaled CO_2 lingers on in traces until the next inspiration, producing higher measurements. As we were interested in CO_2 content of inhaled air under MNC this elevated baseline value exerts a conservative effect, decreasing potential differences. Hence, it cannot invalidate our findings. Even if one were to subtract the difference between our inhaled CO_2 values under baseline, 2.600 ppm, from ambient air content of CO_2 of about 1.000 ppm, i.e .about 0,16% or 1.600 ppm from all measurements to correct for this effect, all our measurements, including that of the child with the lowest value, would still be above the limit of 2.000 ppm.

Another point for potential critique is that we could only exploit every 15th measurement that the apparatus provided. This was due to the fact that we used screen capture as a safe and visual method of capturing the data, as it allowed for immediate feedback about the behavior of the measurement device, and the screen size had to be resized to allow all measurements in one display. But as can be seen from the sample screen (e-Figure 1) the measurements reached stability very soon and a higher frequency of measurements would have produced more stability if anything. But as already those measurements which we took gave very stable results, this limitation does in no way invalidate our results.

We did not see any change in blood-oxygenation, which was measured non-invasively using optical methods. This is likely due to the short time frame of our measurements. This was long enough to demonstrate the rise of CO_2 in the inhaled air, but not to see a change in blood oxygenation. It would be interesting to produce such measurements after prolonged wearing of face masks and when actual symptoms are reported, which we did not see.

The measurement equipment we used is medically certified to measure gases in medically relevant contexts, such as incubators. It has a sensitivity range and a precision that is sufficient for our purpose of CO_2 measurement between 0 and 20 vol%. Although the system has a response delay of 1 second, which rises to 20 seconds if a measurement hose is attached, this does not invalidate our data. We only measured one type of gases at a time, for instance inhaled air. By manually controlling the type of air that was pumped to the measurement sensor during the respective phases, we could make sure that only the type of air that was intended for measurement was directed to the measurement sensor. By disregarding data of a 30 second duration between those phases we allowed for the system to adapt and to make sure that only the type of air intended for a particular measurement phase was considered.

While the measurement apparatus for measuring ambient air is specifically designed for this purpose with a measurement range between 0 and 5.000 ppm, the apparatus used for measuring CO_2 under the mask is designed for a higher measurement range (0-20.000 ppm).

It should be noted that our measurement set-up is similar to that used by the technical gauging of norm values by the German Office of Standards for technical norms of FFP2 masks (DIN EN 149; DIN: Deutsches Institut für Normierung – German Institute of Norms; EN: European Norm). Results of such measurements have led to the current work-place regulations that allow the wearing of FFP2 masks only for 75 minutes, after which a break of 30 minutes is required, exactly because the CO_2 content collects in the mask and the exchange of air is not good enough due to the resistance of the material.

Although we only measured for short periods and every 15 seconds, the short period and the roughly 12 to 15 measurements per period were enough to show how quickly carbon dioxide rises. Considering the fact that children are only rarely affected by SARS-CoV2[17] and that the evidence that they can be asymptomatic carriers is weak [18,19], we suggest that decision makers weigh the hard evidence produced by our experimental measurements accordingly. In our view this would suggest that children should not be forced to wear face masks.

In conclusion we have produced experimental data that show that carbon dioxide content in inhaled air rises up to 13.000 to 13.750 ppm no matter whether children wear a surgical or an FFP2 mask. This is far beyond the level of 2.000 ppm considered the limit of acceptability and beyond the 1.000 ppm that are normal for air in closed rooms. This estimate is rather on the low side, as we only measured this after a short time without physical exertion. Decision makers and law courts should take this into consideration when establishing rules and guidance to fight infections.

Sponsoring

This is an investigator initiated study. There is no external sponsoring, and all members of the study team worked free of charge. MWGFD e.V., a public charity, has organized this study and covered essential expenses only, such as travel expenses.

Conflict of Interest

None of the authors has a conflict of interest.

Data Sharing

Data will be provided publicly after acceptance at https://osf.io/yh97a/?view_only=df003592db5c4bd1ab183dad8a71834f and until then for reviewers or on reasonable request.

Author Contribution Statement

HW is the guarantor of the study. He wrote the protocol, analyzed the data and wrote the first draft of the paper.

RW is one of two medical consultants of the study. He generated the idea, helped organize the study and contributed to data collection, interpretation of the results and writing.

JP was one of the originators of the study, helped organize it, helped with data collection and contributed to writing and interpreting the data.

AD is the second medical consultant. He helped collecting the measurement data and contributed to protocol writing and writing of the paper, as well as to interpreting the data.

HT is the measurement engineer. He provided the technical expertise and collected the measurement data. He produced the original data and helped with data preparation. He also contributed to interpreting and writing.

AK organized the locale for the study, supervised the children and their parents, managed the recruitment process and contributed to data collection, as well as interpretation of the data and writing.

SH is the senior author. He was one of the initiators of the study, provided immunological and general scientific expertise, contributed to organization, data collection, interpretation of the data and to writing.

Ethics Statement

The study was conducted according to the regulations of Helsinki, was submitted to the ethics committee of the University Witten/Herdecke, which approved it under Reg.Nr 2021/22. All children participated at their own free will and could withdraw from the experiment at any time and gave written informed consent, as well as their parents, if children were under age 16.

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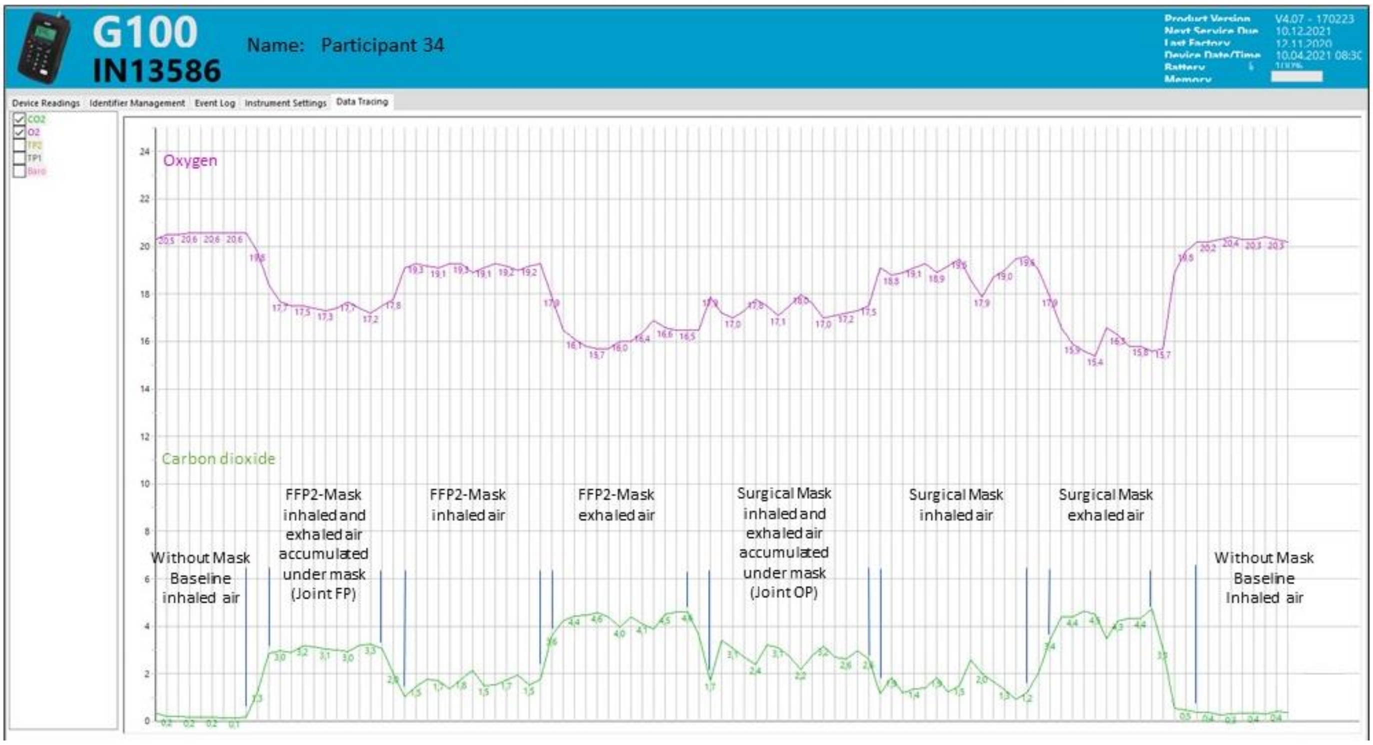
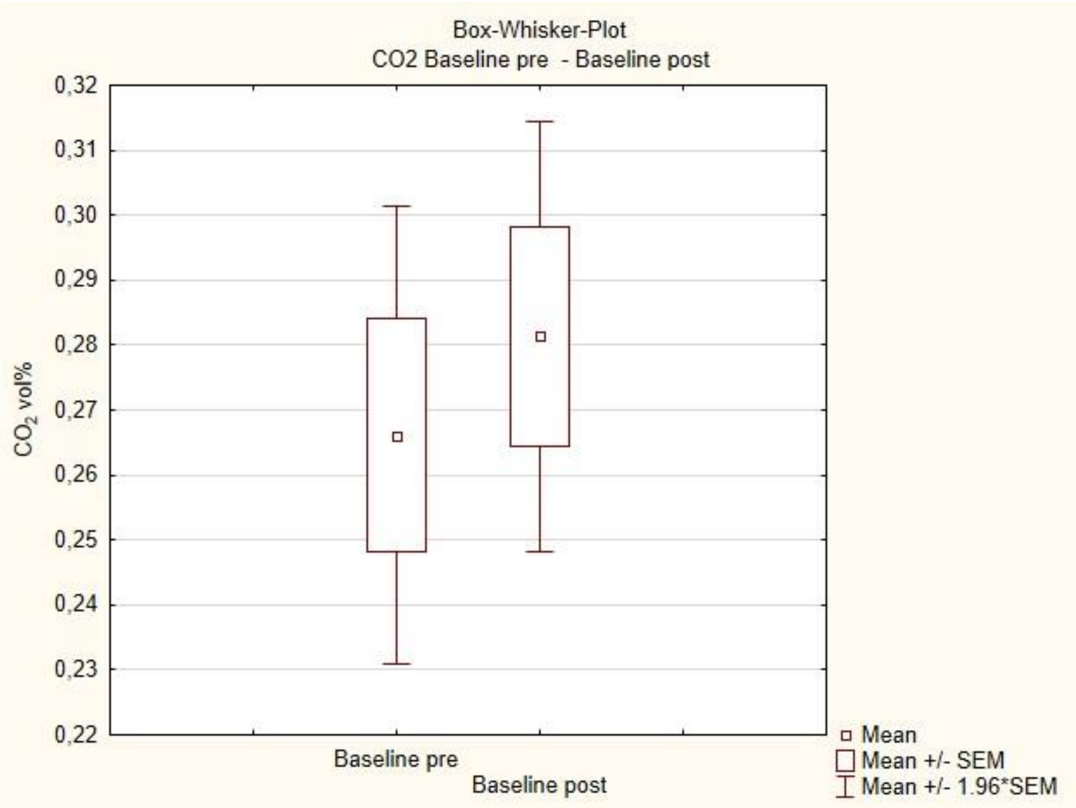
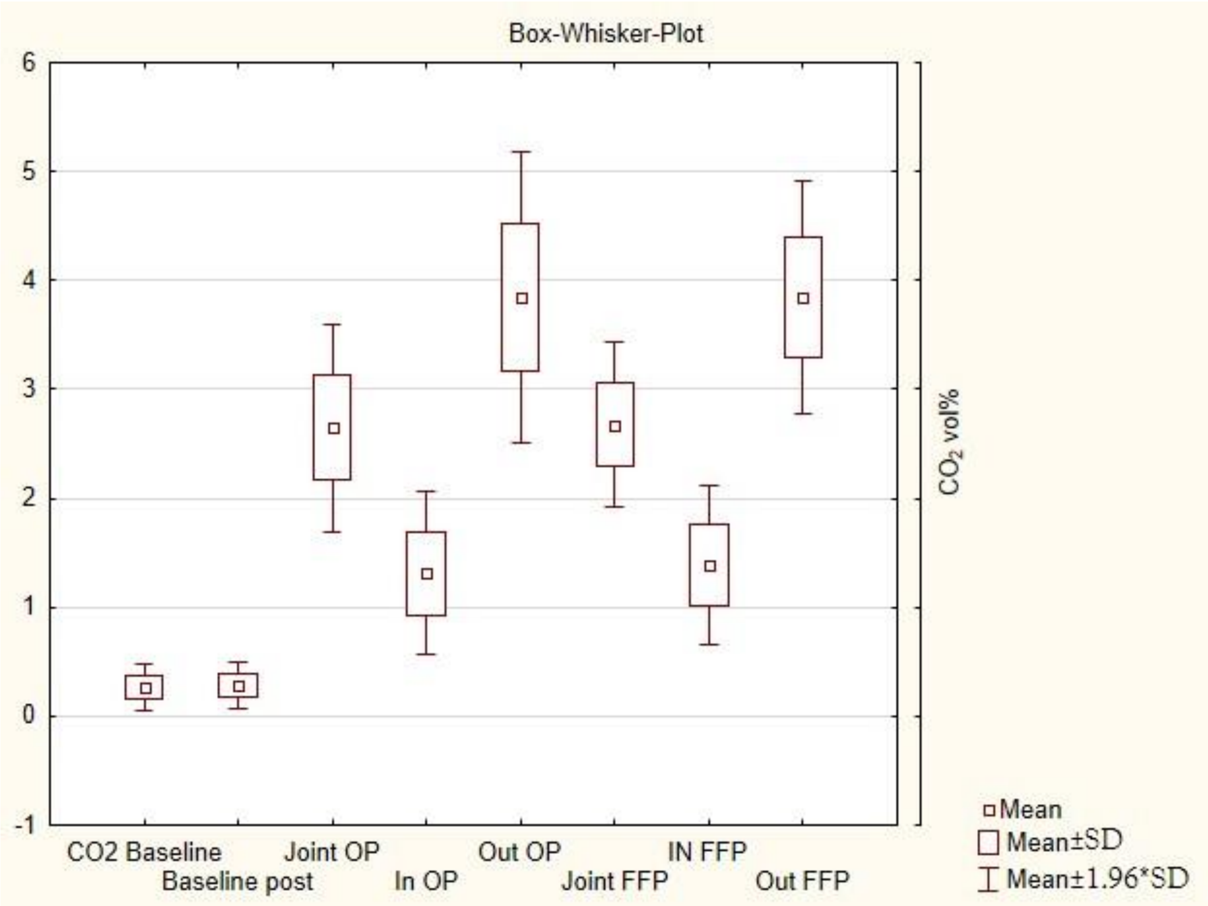
Supplement



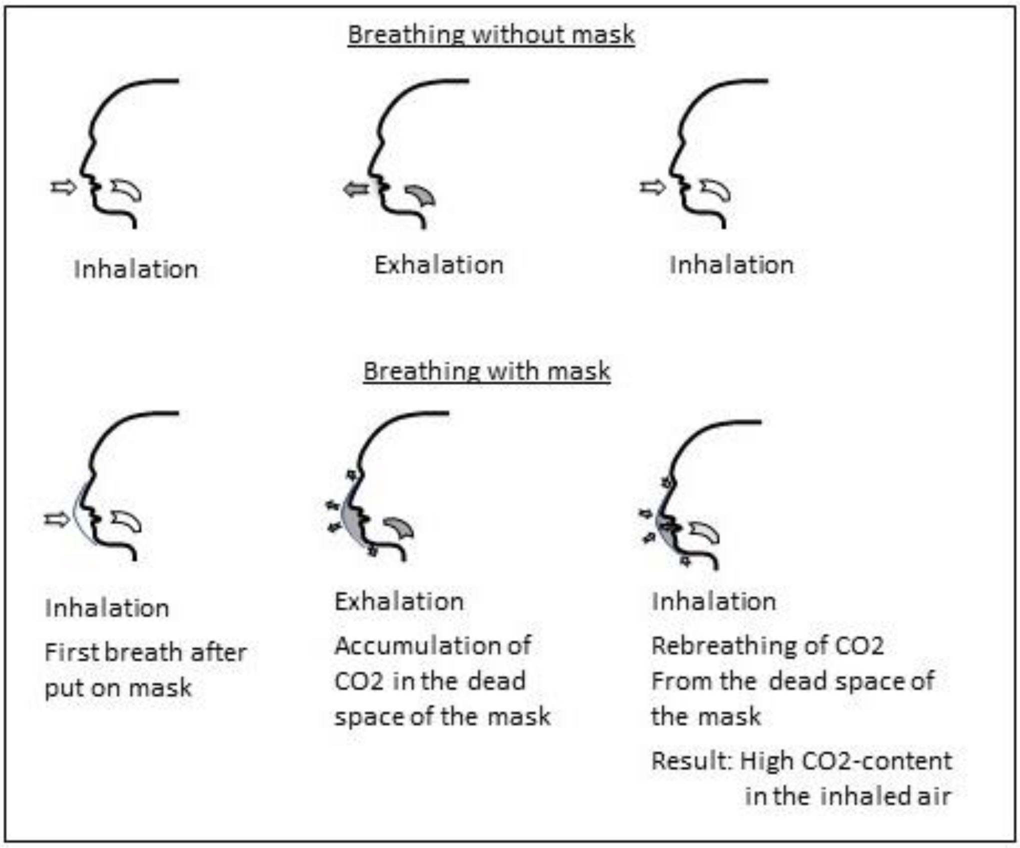
e-Figure 1: Typical sample of a measurement protocol with phases for one child; each bar represents a sampling interval of 15 seconds; measurement unit is vol% of CO_2; 0,1%vol is 1’000 ppm



e-Figure 2 – Baseline and post-baseline measurements; mean CO_2 values (vol%) ± 95% confidence intervals (whiskers)



e-Figure 3 – Box and Whisker Plots of CO_2 values for different measurement phases (Baseline, Baseline post, joint air under surgical mask, inhaled and exhaled air under surgical mask, and joint air, inhaled and exhaled air under FFP masks); given are means, means ± standard deviation and 95% confidence intervals (whiskers)



e-Figure 4 – Breathing without and with mask: the mask provides dead space volume which collects exhaled CO_2 which is then successively mixed with fresh air and re-inhaled

e-Table 1 – Masks and producers used and certifate number

Producer	Mask Type	CE No
Jiandi	KN95 Respirator FFP2	EN 149:2001 + A1:2009
MPG Healthcare	Particle filtering half-mask for children FFP2 LS9688 FFPS NR	0370 EN149:2001 +A1:2009
Schaeffer	MNS surgical mask 3 layered type II	EN 14683
ToyTrade	One-way mask for children	Not available

e-Table 2 – Breathing Frequency and Pulse

	Mean (SD)	n
Breathing Frequency pre	21,87 (4,79)	45
Breathing Frequency Mask 1	23,33 (6,92	45
Breathing Frequency Mask2	22,84 (6,69)	45
Pulse	78,93 (10,76)	43
Pulse post Mask 1	80,12 (10,54)	34
Pulse post Mask 2	82,61 (10,69)	39